19. (Twice Amended) A method for treating nucleic acid, comprising:

a) extracting nucleic acid from a sample suspected of containing one or more microorganisms; and

b) contacting said extracted nucleic acid with an enzymatic cleavage means under conditions such that said extracted nucleic acid forms one or more intra-strand secondary structures, and said cleavage means cleaves said intra-strand secondary structures to produce a plurality of cleavage products.

44. (Twice Amended) A method, comprising:

a) providing:

i) an enzymatic cleavage means in a solution comprising manganese; and

ii) nucleic acid substrate containing microbial gene sequences;

b) treating said nucleic acid substrate with increased temperature under conditions such that substantially single-stranded substrate is produced [said substrate is substantially single-stranded];

c) reducing said temperature under conditions such that said single-stranded substrate forms one or more intra-strand secondary [cleavage] structures;

d) reacting said enzymatic cleavage means with said intra-strand secondary [cleavage] structures so that one or more cleavage products are produced; and

e) detecting said one or more cleavage products.

REMARKS

Claims 1-44 were filed in the original application. Claims 45-54 were added and Claims 2 and 30 were canceled without prejudice in an amendment mailed June 6, 1997.

In the Office Action of August 31, 1998, the Examiner rejected Claims 1, 3-29 and 31-54 under 35 U.S.C. §103(a) as being allegedly obvious under Lyamichev *et al.*, in view of Young, Seela and Roling, and Young *et al.*

THE CLAIMS ARE UNOBVIOUS

The Examiner rejected Claims 1, 3-29, and 31-54 as being allegedly obvious under Lyamichev *et al.,* in view of Young, Seela and Roling, and Young *et al.* The Examiner argues that "[i]n response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (*i.e.*, "the 5' nuclease activity of DNA polymerases may be used to characterize sequence variation between nucleic acids by cleavage of intra-strand secondary structure") are not recited in the rejected claim(s)."

In order to further their business interests and while reserving the right to prosecute broader claims in the future, but without acquiescing to the Examiner's arguments, Applicants have amended independent Claims 1, 19, and 44 to recite that the nucleic acid substrate contains **intra-strand secondary structures** that are targets of the enzymatic cleavage means. None of the references cited by the Examiner (*i.e.*, Lyamichev *et al.,* Young, Seela and Roling, and Young *et al.*), alone, or in combination, teach or suggest characterization of sequence variation or any other applications by cleavage of intra-strand secondary structures. Indeed, as discussed in Applicants' previous Office Action Responses, Lyamichev *et al.,* Young, Seela and Roling, and Young *et al.* teach away from the use of intra-strand secondary structures as targets for cleavage means.

In particular, the PCR methods of Young, Seela and Roling, and Young *et al.* are incompatible with the intra-strand secondary structures of the presently claimed invention.[1] PCR involves the hybridization of a primer to target nucleic acid, wherein secondary structure would confound the hybridization and effectiveness of PCR (*i.e.*, the cleavage structures taught by the presently claimed invention are not compatible with the PCR methods of Young, Seela and Roling, and Young *et al.*).

Additionally, in contrast to the presently claimed invention, Lyamichev teaches the detection of nucleic acids by cleavage of one strand in a structure formed by **inter-strand** annealing (*i.e.,* between a target and a primer) rather than **intra-strand** secondary structures (*See*, Declaration of Mary Ann D. Brow, ¶4).[2] In particular, Lyamichev teaches the use of a

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[1] Discussed in Applicants' Amendment and Response to the Office Action Dated February 17, 1998.

[2] Discussed in Applicants' Amendment and Response to the Office Action Dated February 17, 1998, and Applicants' Amendment and Response to the Office Action Dated January 6, 1997.

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primer for enzyme recognition of cleavage structures for both RNA and DNA substrates. On page 782, middle column, Lyamichev teaches that "[a]n RNA version of the targeted sequence (Fig. 6A, bottom) was cleaved . . . in a reaction that was dependent on the presence of a pilot oligonucleotide . . . ." Thus, for RNA substrates, the cleavage structure of Lyamichev requires formation by **inter-strand** annealing between a target and a primer rather than the intra-strand secondary structures of the presently claimed invention (*See*, Declaration of Mary Ann D. Brow, ¶4). In a telephonic interview held on October 9, 1998, the Examiner conceded that a primer is required in the methods of Lyamichev, when RNA is the nucleic acid substrate.

For DNA substrates, Lyamichev teaches that a primer is required when cleavage reactions are conducted under optimal buffer conditions (*See*, Declaration of Mary Ann D. Brow, ¶4). Specifically, on page 780, column 1, Lyamichev teaches that "[i]n the presence of primer, the rate of cleavage was optimal at about 50 mM KCl (Fig. 3A) . . . In the absence of primer, the maximum rate was at about 20 mM KCl, and the reaction was almost completely inhibited at 50 mM KCl (Fig. 2C, lane 1, and Fig. 3B); also, the reactions (Fig. 3B) were incubated 15 times longer than the reactions shown in Fig. 3A)." Thus, Lyamichev teaches that optimal cleavage is achieved at 50 mM KCl in the presence of a primer. In the absence of primer under these salt conditions, cleavage is almost completely inhibited. Furthermore, even at non-ideal salt concentrations, reactions conducted in the absence of primer had to be incubated **15 times** longer to achieve a similar degree of cleavage as compared to reactions containing primer.

Lyamichev also teaches that a primer is required to control the location of the cleavage event (*See*, Declaration of Mary Ann D. Brow, ¶5). For example, on page 779, Lyamichev teaches that "[i]n the absence of primer, cleavage occurred at the ends of the substrate duplexes (either the extended or shortened forms) between the first and second base pairs," and "[t]he primer-directed shifting of the site of cleavage suggests that precise orientation of the 5' nuclease on the substrate is dominated by the interaction of the polymerization domain of DNAP-*Taq* with the primer." Thus, Lyamichev teaches that a primer is required to control the site of the cleavage. Lyamichev teaches that controlling the site of cleavage with a primer is important in the application of these cleavage methods (*See*, Declaration of Mary Ann D. Brow, ¶6). For example, on page 782, Lyamichev teaches that "[a]n understanding of the 5'

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nuclease activity has several practical consequences. Most exciting is the creation of cleavage reactions that can cut single-stranded polynucleotides in a highly specific manner at **chosen** sequences . . . essentially any sequence can be targeted for cleavage by annealing it to an appropriate pilot oligonucleotide," (emphasis added). On page 782, last column, Lyamichev teaches that the use of primers "greatly increases cleavage efficiency, and reduces the number of unwanted cleavages at regions of secondary structure in the target nucleic acid." Here, Lyamichev explicitly teaches the use of a primer to avoid **unwanted** cleavages at intra-strand secondary structures (*See*, Declaration of Mary Ann D. Brow, ¶6).

Thus, Lyamichev clearly teaches the use of a primer for generating cleavage structures and teaches away from the use of intra-strand secondary structures as targets in cleavage reactions. The cited art fails to establish *prima facie* obviousness because the cited references do not teach or suggest all of the elements of the presently claimed invention (*i.e.*, do not teach or suggest the cleavage of intra-strand secondary structures).[3] Thus, Applicants assert that Claims 1, 2-29, and 31-54 are unobvious over the art cited by the Examiner.

CONCLUSION

For the reasons set forth above, it is respectfully submitted that Applicants' claims as amended should be passed to allowance. Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned collect.

Dated: November 2, 1998

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[3] *See, e.g., Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); and *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).